Trans-NIH Genome-Wide RNAi Screening Program
Status and Initial Call for Proposals
March 10, 2010

Introduction: Gene silencing through RNAi has emerged as a powerful tool for genetic loss-of-function studies. In 2009, the NIH Scientific Directors approved the establishment of a genome-wide RNAi screening facility to perform collaborative projects with intramural investigators, to be housed at the NIH Chemical Genomics Center (NCGC). With funding from NCI (80%) and a consortium of the DDIR with NIAID, NIAMS, NIDCD, NIDDK, NINDS, and NHGRI (combined 20%), a state-of-the-art RNAi screening and informatics facility has now been established. To date, infrastructure and procedures for high throughput screening of human siRNA libraries have been established, including proof-of-principle experiments for assay optimization, compound management, robotics, reader instrumentation, data handling, confirmation, and follow-up biology. While the Facility will not reach full capacity until FY2011, this memo announces the provisional opening of the Facility, for the purpose of beginning solicitation of potential projects, either for assistance with assay development, or for screening project initiation if appropriate.

Scientific and operational specifics follow, followed by project requirements and the application process.

Governance headed by: Bob Wiltrout and Michael Gottesman

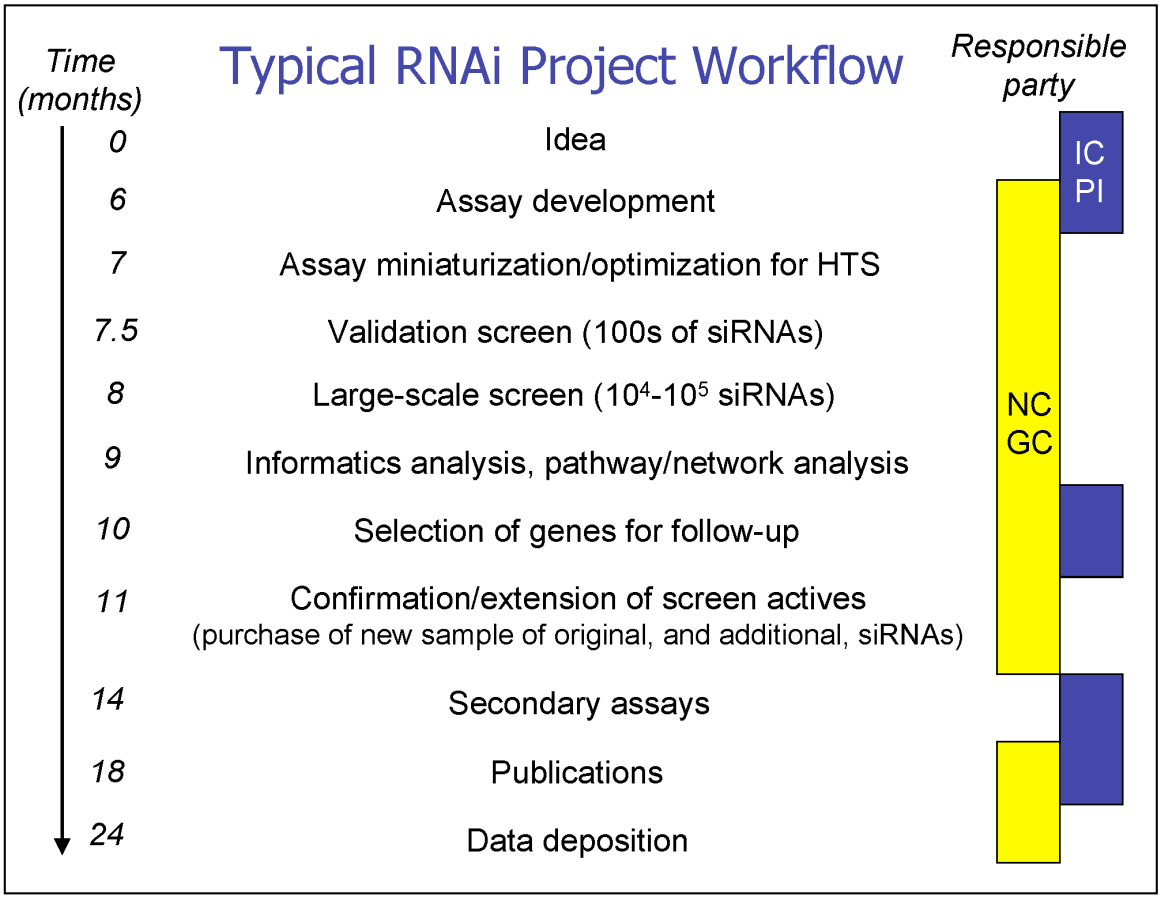
Scientific POC/Head of RNAi Project Team: Scott Martin, NCGC

Type of RNAi performed currently: human siRNA and miRNA only; shRNAs and other species capacities may be added in the future depending on demand

Libraries: siRNA kinome (several suppliers), siRNA druggable genome, miRNA mimic; whole genome and miRNA inhibitor libraries to be added later in FY2010

Scale of projects to be performed: projects must aim to screen at least the "druggable genome" ≥6,500 genes to be eligible for running at NCGC. Projects below this scale can be conducted within individual labs. NIH-wide specific pricing should be available from most vendors of RNAi related reagents; further details can be obtained from the vendor and in most cases through the NIH Intramall site (examples of vendors include, though are not limited to, Ambion/Applied Biosystems, Dharmacon/Thermo Scientific, Invitrogen, Qiagen and Sigma).

Project scope/deliverables: the desired endpoint of all projects is important insight(s) into a pathway or phenotype, and one or more joint publications. Each project has multiple stages (see Figure below), with varying degrees of shared responsibility between NCGC and the collaborating PI. The average project lasts 18-24 months, with the screen itself taking up a small part of this time; the time-consuming, difficult, and ultimately most important part of any project is the work before and after the screen.



Given this substantial commitment of time and resources, approval via formal application will be required for projects to be performed (see below). However, since investigators are frequently unfamiliar with the requirements of this type of large-scale screening project, *investigators are strongly encouraged to discuss proposals with the RNAi project team (contact: Scott Martin, martinsc@mail.nih.gov) prior to submission*. The RNAi Project Team can assist with all levels of project development. Hands-on assistance and access to equipment can be provided to facilitate optimization. In addition, in the case of systems not amenable to large-scale screening, the Project Team will provide advice on strategies and commercially available reagents for conducting smaller, more focused screens in their own labs.

In addition to robust assays, lipid-based transfection and siRNA controls are also critical for large-scale siRNA screening. Applicants must demonstrate siRNA-mediated gene silencing in their model systems prior to submission. Transfected siRNAs should yield efficient knockdown with limited side effects. The Project Team can recommend validated reagents and protocols for these types of experiments. If a system proves refractory to lipid-based transfection, and alternative model systems cannot be employed, access to medium throughput electroporation may be provided to facilitate investigation. Investigators with assays lacking robust positive control siRNAs should specify candidate genes whose knockdown may affect the biology under investigation. The Project Team can provide access to corresponding reagents for evaluation. Small-scale screens can also be conducted to facilitate control identification.

Once a project is formally accepted, the RNAi Project Team will further refine conditions and conduct pilot screens. Upon completion, a full scale campaign will be conducted and actives will

be confirmed in follow-up experiments. The Project Team will apply extensive informatics to rank and enrich hit lists with associated data, such as pathway information and GO term enrichments. In many cases, these lists will contain large numbers of genes. Investigators will need to specify robust secondary assays to help prioritize hits within these lists. The entire process is intended to be highly collaborative, resulting in joint publications and eventual data release. As such, investigators should identify personnel that will be dedicated to the project.

Application Process: applications will go through three stages of review.

1. IC SD: the investigator should submit the proposal to his/her SD, who will initially review for scientific merit and relevance to IC mission
2. RNAi Proposal Review Committee: will rank proposals based on novelty, importance to one or more fields of science, and technical characteristics of primary screen and available follow-up assays
3. Final selection will be performed by Drs. Wiltrout and Gottesman, as primary funders of the RNAi initiative.

As outlined in the application, a number of technical criteria need to be addressed prior to project approval. Projects must have cell-based model systems and an assay amenable to high-throughput screening. The RNAi Project Team can accommodate a wide variety of assay formats, ranging from luminescence and fluorescence-based assays to high content imaging. Assays must exhibit good Z'-factors and CVs in microplate format (e.g. 96 or 384 well). The Project Team can advise on these criteria and strategies to improve existing assays.

Following internal institute(s) review and approval, the Trans-NIH RNAi Proposal Review Committee will consider whether an application should proceed, or be returned to the applicant for further scientific and/or technical refinement. Key criteria to be considered by the committee are likely scientific impact, novelty, presence of appropriate technical requirements, and the plan for and commitment to follow-up. Please contact the RNAi Project Team directly for technical advice and assistance with project development and financial estimates prior to submission.

Application form is attached.

FOR FURTHER INFORMATION/INQUIRIES:

Primary contact (project ideas/questions/advice):
Scott Martin, RNAi Project Team Head, NCGC; martinsc@mail.nih.gov; 301-217-1079

NCGC Director: Chris Austin; austinc@mail.nih.gov; 301-217-5733

Project Selection Committee Chair: Natasha Caplen, NCI; ncaplen@mail.nih.gov; 301-451-1844

Application for RNAi Project Collaboration

Name:

Title:

IC/Branch:

Date:

Abstract: Less than 250 words.

Target/Pathway/Cellular Phenotype is to be assayed:

Desired scope of screen (e.g., druggable genome, whole genome)

Species:

Prior RNAi screens, of any scale, conducted in similar systems:

Specific Aims/Desired deliverables of Project:

Primary Assay: Describe

- The high-throughput amenable assay, including Z'-factors and CVs in 96-well format.
- Reagents necessary for the assay and their availability and cost. Note those that may be difficult or expensive to obtain in sufficient quantities.
- Preliminary data and controls using siRNAs and/or compounds
- Data on cell transfectability and method thereof

Secondary Assays: Describe secondary assays for hit prioritization and follow-up. These should include both assays at the same level of biological complexity with alternative readouts, and more physiological, lower-throughput assays to assess biological robustness and relevance.

Follow-up Plan: Describe follow-up experiments and applications to be conducted, with endpoint of high quality publications and data useful to the research community

Personnel: Indicate personnel that will be committed to project over the expected two-year duration of the project

Estimated Costs:

Applicants: Submit completed form to your IC SD for review

Indication of review and approval by applicant's SD: